MOLECULAR AND CELLULAR BIOLOGY, Jan. 1988, p. 1–9
0270-7306/88/010001-09$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 8, No. 1

Comparison of Nonerythroid α-Spectrin Genes Reveals Strict Homology among Diverse Species

THOMAS L. LETO,[1]†* DONNA FORTUGNO-ERIKSON,[1] DAVID BARTON,[2] TERESA L. YANG-FENG,[2] UTA FRANCKE,[2] ALAN S. HARRIS,[1] JON S. MORROW,[1] V. T. MARCHESI,[1] AND EDWARD J. BENZ, JR.[2,3]

Departments of Pathology,[1] Human Genetics,[2] and Internal Medicine,[3] Yale University School of Medicine, New Haven, Connecticut 06510

Received 1 June 1987/Accepted 22 September 1987

The spectrins are a family of widely distributed filamentous proteins. In association with actin, spectrins form a supporting and organizing scaffold for cell membranes. Using antibodies specific for human brain α-spectrin (α-fodrin), we have cloned a rat brain α-spectrin cDNA from an expression library. Several closely related human clones were also isolated by hybridization. Comparison of sequences of these and other overlapping nonerythroid and erythroid α-spectrin genes demonstrated that the nonerythroid genes are strictly conserved across species, while the mammalian erythroid genes have diverged rapidly. Peptide sequences deduced from these cDNAs revealed that the nonerythroid α-spectrin chain, like the erythroid spectrin, is composed of multiple 106-amino-acid repeating units, with the characteristic invariant tryptophan as well as other charged and hydrophobic residues in conserved locations. However, the carboxy-terminal sequence varies markedly from this internal repeat pattern and may represent a specialized functional site. The nonerythroid α-spectrin gene was mapped to human chromosome 9, in contrast to the erythroid α-spectrin gene, which has previously been assigned to a locus on chromosome 1.

The spectrins are filamentous proteins found in the cortical cytoplasm of eucaryotic cells. Although spectrin was initially identified as a structural component unique to erythrocytes, recent work has identified immunologically cross-reactive counterparts in most nonerythroid tissues (for reviews, see references 2 and 23). Preliminary studies of the nonerythroid spectrins suggest that these proteins are membrane skeleton components with functional activities similar to those described for erythrocyte spectrin. These include calmodulin binding, cross-linking of actin filaments, and attachment to membrane receptors (3, 13, 20, 26, 28). The proteins that have been characterized appear to be long, rodlike molecules composed of two nonidentical subunits. Avian spectrins share a common α-chain of M_r 240,000 that associates with a number of different tissue-specific β-subunits (14). The mammalian erythroid and nonerythroid α-chains are similar in molecular weight, but are structurally distinct and share only weak immunological cross-reactivity (14, 16, 27). In this paper we report the results of structural comparison of a spectrin derived from the molecular cloning of several cDNAs encoding carboxy-terminal portions of the major mammalian nonerythroid α-spectrin (α-fodrin). Analysis of these clones has enabled a direct comparison to be made of the primary structures of α-spectrin in several species and tissues.

MATERIALS AND METHODS

Isolation of cDNA clones. Screening for cDNA recombinant DNA bacteriophages expressing nonerythroid α-spectrin with antibodies involved the use of a λgt11 library constructed from mRNA derived from 1- and 2-week-old rat brains (generously provided by N. Davidson and A. Dowsett, California Institute of Technology). *Escherichia coli* Y1090 cells, infected with these phage, were used as the host and plated at a density of 3×10^4 PFU/150-mm plate. Procedures used for growth, induction, and antibody screening of recombinant phage have been previously described (33, 34). The antibody used has been previously characterized (16) and shown to react primarily against the α-subunit of brain spectrin (α-fodrin). Prior to use, 5 ml of antiserum was diluted 1:50 with 50 mM Tris hydrochloride (pH 8)–150 mM NaCl–3% bovine serum albumin and preabsorbed with 2 ml of a boiled Y1090 bacterial cell lysate. This reagent was capable of detecting 10 ng of purified human fodrin spotted onto nitrocellulose under the conditions used for screening. Bound antibodies were detected with ^{125}I-labeled *Staphylococcus aureus* protein A (10^6 cpm per filter) as previously described (33, 34).

The immunoreactive rat brain cDNA clone (RBF2) was used to infect and lysogenize *E. coli* Y1089 cells (33). The inducible galactosidase anti-α-fodrin immunoreactive fusion protein, produced by these lysogens, was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting procedures (33).

The *Eco*RI-digested insert of RBF2 (2.5 kilobases [kb]) was used as a hybridization probe to screen a human neuroblastoma cDNA library (19) (a generous gift of F. W. Alt, Columbia University) constructed in Charon 16a. The ^{32}P-labeled RBF2 probe (specific activity, $>5 \times 10^8$ cpm/μg) was synthesized with random hexamer oligonucleotide primers as described previously (7). Hybridization screening was performed in 50% formamide solution at 42°C for 20 h, followed by washes in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7]) – 0.1% sodium dodecyl sulfate at 37°C and in 0.2× SSC–0.1% sodium dodecyl sulfate at 50°C.

DNA sequencing. All *Eco*RI-derived insert cDNA fragments were subcloned into M13 vectors (mp18 and mp19) for sequencing. The DNA sequence was obtained from both strands by using standard dideoxynucleotide-terminated reaction procedures (24). Interior portions of cDNAs were

* Corresponding author.
† Present address: Section of Bacterial Infectious Disease, National Institute of Allergy and Infectious Diseases, Bethesda, Md. 20892.

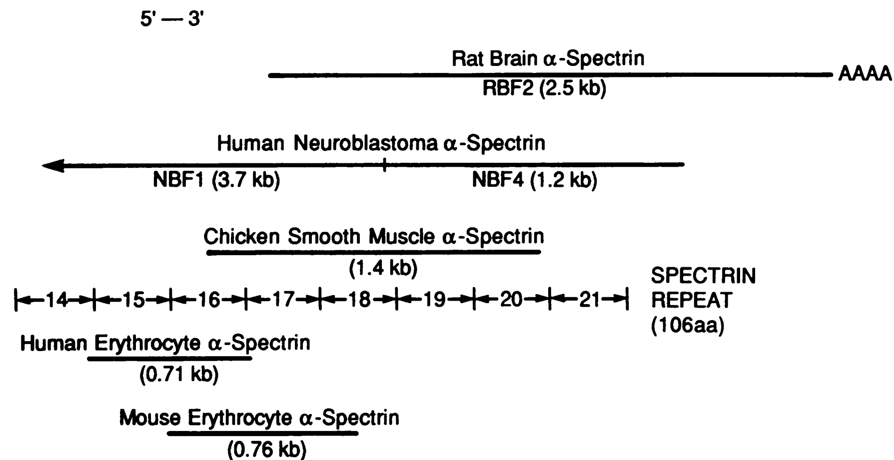

FIG. 1. Alignment of homologous nonerythroid and erythroid α-spectrin cDNAs. The assignment of repeat numbers (106 amino acids in length) is based on comparison with previously assigned repeats sequenced in erythrocyte α-spectrin (5, 30, 31). The rat nonerythroid α-spectrin (RBF2) cDNA (2.5 kb) contains sequence encoding repeats 17 to 21, followed by approximately 1 kb of untranslated sequence and a poly$(A)^+$ 3' terminus. The homologous human neuroblastoma cDNAs, NBF1 (3.7 kb) and NBF4 (1.2 kb), selected by hybridization screening with RBF2 as a probe, share a common 3' terminus. The relative positions of the human and rat sequences, as well as previously published chicken smooth-muscle (4, 32) and mouse and human erythroid (10) sequences, were determined by using the University of Wisconsin Genetics Computer Group Bestfit analysis program. Calculated homologies between all genes and predicted protein structures are given in Table 1.

sequenced from a series of deleted M13 subclones which were generated by T4 DNA polymerase exonuclease digestions as described previously (6). Sequences were compared and aligned by using the sequence analysis software package of the University of Wisconsin Genetics Computer Group.

Chromosome mapping. Chromosome mapping was done first by Southern blot analysis of DNA from Chinese hamster × human somatic hybrid cell lines (9–12). *Bgl*II-digested DNA (10 μg per lane) was separated by electrophoresis, transferred to nitrocellulose, and hybridized with rat brain α-spectrin probe (RBF2) or the human probe (NBF1) as described previously (1). The RBF2 probe was labeled as described previously (7). The human neuroblastoma α-spectrin cDNA probe was synthesized from a single-stranded template of NBF1 which was subcloned into the vector M13 as described previously (24). Faint bands were confirmed by longer exposure times. The chromosome locus was independently assigned by direct in situ hybridization to human metaphase chromosomes (15). In these studies the probe was prepared from RBF2 (2.5-kb insert) labeled by nick translation with $[^3H]$dCTP, $[^3H]$dTTP, and $[^3H]$dATP.

RESULTS AND DISCUSSION

The initial cloning involved the use of antibodies reactive primarily against the major isoform of human brain α-spectrin (16) to probe a rat brain cDNA library constructed in the expression vector λgt11 (33, 34). One immunopositive clone was selected from a screening of 8×10^5 recombinants. This clone, designated RBF2, contained a 2.5-kb cDNA insert and was used as a hybridization probe to screen a human neuroblastoma cDNA library (19), from which several candidate clones were selected. These human cDNAs ranged in size from 1.2 to 3.7 kb.

The human and rat cDNA inserts were subcloned into the bacteriophage M13 and sequenced (6, 24). The alignment of these genes with the carboxy-terminal portion of the α-chain (Fig. 1) was based on the following information. Clone RBF2 contained one long open reading frame encoding 475 amino acids followed by 1 kb of untranslated sequence and a poly $(A)^+$ 3' terminus. Galactosidase induction of strain Y1089, transfected with the RBF2 phage, led to the expression of a galactosidase- anti-brain-α-spectrin reactive fusion protein of a size consistent with a sequence encoding the carboxy-terminal 50 kilodaltons of the α-subunit. The human clones (NBF1 and NBF4) shared a common 3' terminal sequence and were identical throughout the region of common overlap, except for 50 base pairs at the 5' terminus of NBF4, which showed an abrupt departure from this pattern and was presumed to be a cloning artifact. The peptide sequence deduced from the larger human clone, NBF1, aligned exactly with a 21-residue peptide derived from a terminal tryptic fragment of human brain α-spectrin (A. S. Harris, S. Keithan, D. W. Speicher, and J. S. Morrow, unpublished data), thus confirming that these cDNAs encode the predominant isoform of nonerythroid α-spectrin. The rat and human cDNAs exhibited extensive homology throughout their overlapping sequences. Comparison of both these genes with another previously reported spectrin gene, cloned from chicken gizzard cDNA (4, 32), revealed the same striking pattern of strict structural homology within this protein

FIG. 2. Homology among nonerythroid α-spectrins. (a) Nucleotide (A to C) and deduced amino acid (A' to C') sequences for human, rat, and chicken (4, 32) nonerythroid α-spectrins. Only rat and chicken sequences which differ from human sequences are given. Bases are numbered starting at the 5' end of clone RBF2. (b) Two-dimensional matrix comparison (22) of deduced peptide sequences from rat and chicken α-spectrin. With the homology search program COMPARE (University of Wisconsin Genetics Computer Group), matches of 8 or more identical residues in a window of 30 were scored. The boundaries of repeats were based on homology to repeating units defined in erythroid spectrin (30, 31). Repeats 20 and 21 have diverged from the regular 106-amino-acid repetitive pattern and display the poorest homology with other internal repeats.

```
a                 10                  30                  50                  70                  90                  110
                   .         .         .         .         .         .         .         .         .         .         .         .
A (MAN)   CCGGGACATGGATGACGAGGAGTCCTGGATCAAGGAGAAGAAACTGCTGGTGGGCTCAGAGGACTACGGCCGGGACCTAACTGGTGTGCAGAACCTGAGGAAGAAGCACAAGCGGCTGGA
B (RAT)      A                                      GT  T A        T        T   A A     C           A   T          A            A
C (CHKN)     T           T G               A            T                   T   A A     G                          A   T     CT

A'(MAN)    R  D  M  D  D  E  E  S  W  I  K  E  K  K  L  L  V  S  S  E  D  Y  G  R  D  L  T  G  V  Q  N  L  R  K  K  H  K  R  L  E
B'(RAT)
C'(CHKN)

                130                 150                 170                 190                 210                 230
                  .         .         .         .         .         .         .         .         .         .         .         .
A         AGGCAGACTGGCTGCGCATGAGCCGGCTATTCAGGGTGTCCTGGACACTGGCAAGAAGCTGACCGATGACAACACCATCGGGAAAGAGGAGATCCAGCAGCGTCTGGCGCAGTTTGTGGA
B                    C  A  C  A  A  C                    G  G         TT                   C G                     C A
C          CAGA T A     C     A  T     C        T  A                 TT A     T     A  T     G        A      AA      T GA

A'        G  R  L  A  A  H  E  P  A  I  Q  G  V  L  D  T  G  K  K  L  T  D  D  N  T  I  G  K  E  E  I  Q  Q  R  L  A  Q  F  V  E
B'                                                                    S                    Q
C'        A  E                                                        S                                               R        D

                 250                 270                 290                 310                 330                 350
                   .         .         .         .         .         .         .         .         .         .         .         .
A         GCACTGGAAAGAGCTGACAGAGCTGGCAGCTGCCCGGGGTCAGCGGCTGGAAGAGTCCTTGGAATATCAGCAGTTTGTAGCCAATGTGGAAGAGGAAGAAGCCTGGATCAATGAGAAAAT
B                  G  A    A C    A        A     C           G           G              G     C     G     G  G  T              G
C         C            T A A C             T     G     T     G      C    G  C  A           A     T  G  A  G  G  A        C

A'         H  W  K  E  L  T  E  L  A  A  A  R  G  Q  R  L  E  E  S  L  E  Y  Q  Q  F  V  A  N  V  E  E  E  E  A  W  I  N  E  K  M
B'                        K  Q
C'                        K  Q

                 370                 390                 410                 430                 450                 470
                   .         .         .         .         .         .         .         .         .         .         .         .
A         GACCCTGGTGGCCAGCGAAGATTATGGCGACACTCTTGCCGCCATCCAGGGCTTACTGAAGAAACATGAAGCTTTTGAGACAGACTTCACTGTCCAGAAGGATCGCGTGAATGATGTCTG
B                              C  C  G           T                                                        C        A
C            AT    A     T  G        A     A     T  T           G        G        A  C     T     T        C  A  CA A           T

A'         T  L  V  A  S  E  D  Y  G  D  T  L  A  A  I  Q  G  L  L  K  K  H  E  A  F  E  T  D  F  T  V  Q  K  D  R  V  N  D  V  C
B'                                                                                                      H
C'                                                                                                      H

                 490                 510                 530                 550                 570                 590
                   .         .         .         .         .         .         .         .         .         .         .         .
A         TACCAATGGACAAGACCTCATTAAGAAGAACAATCACCATGAGGAGAACATCTCTTCAAAGATGAAGGGCCTGAACGGGAAAGTGTCAGACCTGGAGAAAGCTGCAGCCCAGAGAAAGGC
B            T                                                                 T     T  T        T              A     T     G  A
C         CG T      G G  T        A     T          T         TA  G T              C  G  C  G  A     T           C     T     G  A

A'         T  N  G  Q  D  L  I  K  K  N  N  H  H  E  E  N  I  S  S  K  M  K  G  L  N  G  K  V  S  D  L  E  K  A  A  A  Q  R  K  A
B'
C'         A        E                             V           T  A
```

```
             610                 630                 650                 670                 690                 710
               .         .         .         .         .         .         .         .         .         .         .         .
A     GAAGCTGGATGAGAACTCGGCCTTCCTTCAGTTCAACTGGAAGGCGGACGTGGTGGAGTCCTGGATCGGTGAAAAGGAGAACAGCTTGAAGACAGATGATTATGGCCGAGACCTGTCTTC
B                                         T        T                    T                       A                    T
C     C  A              T        C              A  A  T           A     A     G     A     TC                      T     C  C

A'     K  L  D  E  N  S  A  F  L  Q  F  N  W  K  A  D  V  V  E  S  W  I  G  E  K  E  N  S  L  K  T  D  D  Y  G  R  D  L  S  S
B'
C'

             730                 750                 770                 790                 810                 830
               .         .         .         .         .         .         .         .         .         .         .         .
A     TGTGCAGACGCTCCTCACCAAACAGGAAACTTTTGACGCTGGGCTGCAGGCCTTCCAGCAGGAAGGCATTGCCAACATCACTGCCCTCAAAGATCAGCTTCTCGCCGCCAAACACGTTCA
B        C  A  T  G        G     G  A     T     C                    G        A  T                 C     G  A  T     G   A
C           A  A  A                 C     T     A  T     T           G  A     A           T  G     C     A  G  A        TA C

A'     V  Q  T  L  L  T  K  Q  E  T  F  D  A  G  L  Q  A  F  Q  Q  E  G  I  A  N  I  T  A  L  K  D  Q  L  L  A  A  K  H  V  Q
B'                                                                                                                       I
C'                                                                                                                       I

             850                 870                 890                 910                 930                 950
               .         .         .         .         .         .         .         .         .         .         .         .
A     GTCCAAGGCCATCGAGGCCCGGCACGCCTCCCTCATGAAGAGGTGGAGCCAGCTTCTGGCCAACTCAGCCGCCCGCAAGAAGAAGCTTCTGGAGGCTCAGAGTCACTTCCGCAAGGTTGG
B        G                 A  T                      C      GT C  T  T  T  AA              T G  A     C        T     A     A A
C     A  A        T    TT  T     T  T  G     AC C    AT     G  T  T  T  T  A     A  G  A  A  CT           GAG       AA       A

A'     S  K  A  I  E  A  R  H  A  S  L  M  K  R  W  S  Q  L  L  A  N  S  A  A  R  K  K  K  L  L  E  A  Q  S  H  F  R  K  V  G
B'                                                    T        F              T                                               E
C'                    V                             N                                                     E                 E

             970                 990                1010                1030                1050                1070
               .         .         .         .         .         .         .         .         .         .         .         .
A     AGGACTCTTCCTGACCTTCGCCAAAAAGGCTTCTGCCTTCAACAGCTGGTTTGAAAATGCAGAGGAGGACTTAACAGACCCCGTGCGCTGCAACTCCTTGGAAGAAATCAAAGCTTTGCG
B       AC         G    T           A  G  T                 G        A      C C        A              TC                CC C
C       AT      T    T  T     G     C              T        G     T         C G  G  T              T   C                AC

A'     G  L  F  L  T  F  A  K  K  A  S  A  F  N  S  W  F  E  N  A  E  E  D  L  T  D  P  V  R  C  N  S  L  E  E  I  K  A  L  R
B'     D           A
C'     D

            1090                1110                1130                1150                1170                1190
               .         .         .         .         .         .         .         .         .         .         .         .
A     CGAGGCCCACGACGCCTTCCGCTCCTCCCTCAGCTCTGCCCAGGCTGACTTCAACCAGCTGGCCGAGCTGGACCGCCAGATCAAGAGCTTCCGCGTAGCCTCCAACCCCTACACCTGGTT
B     A     T  T  T           A  G           G     C              A              T           T     A  G        T
C     A  A        T        T     A  T           A            ]← 3' END OF CHICKEN α-SPECTRIN

A'     E  A  H  D  A  F  R  S  S  L  S  S  A  Q  A  D  F  N  Q  L  A  E  L  D  R  Q  I  K  S  F  R  V  A  S  N  P  Y  T  W  F
B'
C'
```

```
          1210                1230                1250                1270                1290                1310
A   TACCATGGAGGCCCTGGAGGAGACCTGGAGGAACCTACAGAAAATCATCAAGGAGAGGGAGCTGGAGCTGCAGAAGGAACAGCGGCGGCAGGAGGAGAACGACAAGCTGCGCCAGGAGTT
B   C                 A     G                 G     T      C A  A                                       T        A     A

A'   T  M  E  A  L  E  E  T  W  R  N  L  Q  K  I  I  K  E  R  E  L  E  L  Q  K  E  Q  R  R  Q  E  E  N  D  K  L  R  Q  E  F
B'

          1330                1350                1370                1390                1410                1430
A   TGCCCAGCACGCCAACGCCTTCCACCAGTGGATCCAAGAGACCAGGATAACTCTCCTCGATGGGTCCTGTATTGGTGGAAGAGTCGGGGACTCTCGAATCCCAGCTTGAAGCTACCAAAC
B            T        G                 G  A  A  A CGTA         C        C      C           A     G     T

A'   A  Q  H  A  N  A  F  H  Q  W  I  Q  E  T  R  I  T  L  L  D  G  S  C  I  G  G  R  V  G  D  S  R  I  P  A ] ⇐ CARBOXYL
B'                                                T  Y                          R           N     G     S    ]   TERMINUS

          1450                1470                1490                1510                1530                1550
A   GCAAGCACCAGGAAATCCGAGCCATGAGAAGTCAGCTCAAAAAGATCGAGGACCTGGGGGCCGCCATGGAGGAGGCCCTCATCCTGGACAACAAGTACACGGAGCACAGCACCGTGGGCC
B                GTG                 G  G     T              T           A                          T           T

          1570                1590                1610                1630                1650                 Eco RI
A   TCGCCCAGCAGTGGGACCAGCTGGACCAGCTGGGCATGCGCATGCAGCACAACCTGGAGCAGCAGATCCAGGCCAGGAACACAACAGGTGTGACTGAGGAGGCCCTCAAAGAATTC
B    G                  T A                                                                 A  C                 G  G
                                                                    3' END OF HUMAN NEUROBLASTOMA α-SPECTRIN ↑
```

b

RESIDUE NUMBER

CHICKEN

RAT

REPEAT NUMBER

96 % Homology

TABLE 1. Summary of homologies among nonerythroid and erythroid α-spectrins[a]

α-Spectrin	% Homology in:		% Nucleotide Replacements	
	Nucleotides	Proteins	Syn	Nonsyn
Nonerythroid				
Rat versus human	89	97	10.1	1.1
Rat versus chicken	81	96	17.2	1.8
Human versus chicken	82	95	16.3	1.8
Erythroid				
Human versus mouse	82	83	11.4	6.7
Nonerythroid versus erythroid				
Human repeat 16		52		
Human repeat 19		54		
Rat versus mouse		66		

[a] Homologies are expressed as percent identities; mutations are designated as either synonymous (Syn) or nonsynonymous (Nonsyn). Calculations are derived from the regions of common overlap illustrated in Fig. 1.

family. A representative comparison of these genes and the closely homologous proteins which they encode is given in Fig. 2a. This analysis demonstrated that the majority of base changes occurred at the third position of degenerate codons. Thus, the chicken and rat cDNA sequences, which are 81% homologous, encode proteins which are greater than 96% identical. The few nonsynonymous mutations observed between species were predominantly conservative amino acid substitutions and were uniformly dispersed throughout the entire lengths of overlapping sequences. Thus, minimal structural and functional differences between species are predicted. These strict homologies stand in marked contrast to the divergence seen among erythroid spectrins of different species. Recent data derived from erythroid α-spectrin cDNA clones (5) show a significantly greater frequency of interspecies nonsynonymous base replacements. Thus, mouse and human erythrocyte α-spectrin cDNAs, which are 82% homologous, predict proteins that are only 83% identical. The data in Table 1, which summarize these and other patterns of homology, illustrate how rapidly the mammalian erythroid α-spectrins have evolved at the amino acid level relative to their more highly conserved nonerythroid counterparts.

A distinctive property of erythrocyte spectrin, consistent with its rodlike shape, is its repetitive internal structure (30, 31). Peptide sequences derived from both α- and β-subunits were compatible with a common structural motif: both chains were composed of multiple 106-amino-acid repeating units which were predicted to fold into triple-helical segments interrupted by turns and nonhelical connecting regions. The same repetitive structure was noted in the peptide sequence deduced from the chicken smooth-muscle cDNA

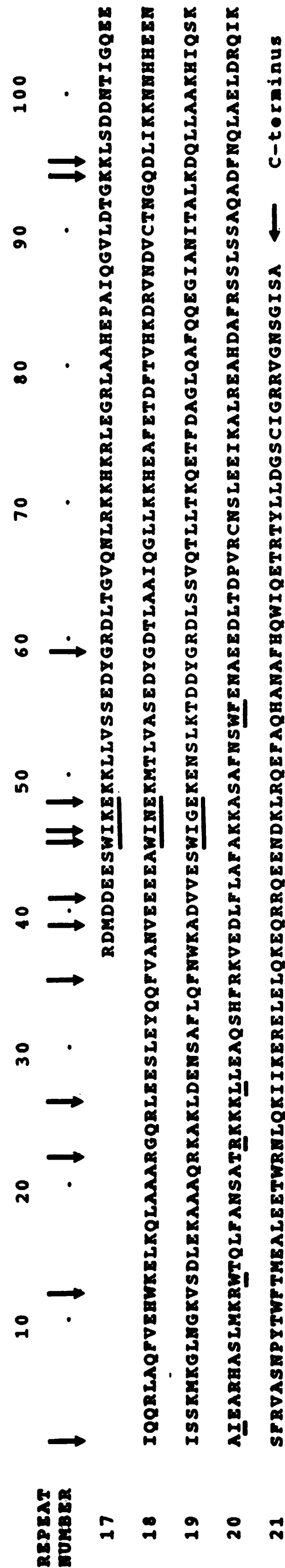

FIG. 3. Homologous internal repeat structure of rat brain α-spectrin. The origin of each repeat is defined by homology to erythroid structures (30, 31). Arrows above the sequences designate most strongly conserved residues characteristic of erythroid spectrin homologous repeats. Two frame shifts (one residue in the first half and several residues in the second half) are required to align some highly conserved residues (underlined) in repeat 20. Carboxy-terminal repeat 21 shows greatest variance and requires an additional frame shift to align the conserved cluster WIQE (residues 45 to 48) in register with the other repeats.

(4, 32) and was also found to be a feature of the mammalian nonerythroid α-spectrin sequences reported here.

Pairwise comparison of homology between the sequences of chicken and rat nonerythroid α-spectrins by two-dimensional matrix analysis (Fig. 2b) illustrates this same internal repeat structure characteristic of all spectrins. Like erythroid spectrin, the nonerythroid α-spectrins showed greatest internal homology within the first two-thirds of the repeating unit. Most α-subunit repeats show a regular periodicity of 106 amino acids. However, repeats 20 and 21 are less conserved and require shifts to align residues in phase with the most highly conserved residues in other repeats (Fig. 3). Despite these deviations from the normal repeat structure, the carboxy-terminal region of the nonerythroid α-spectrin is as strictly conserved across species as are the other internal repeats farther upstream (Fig. 2a). The variability in repeats 20 and 21 may represent alterations involved in a specialized and important function, such as actin filament binding, which occurs in this region and has remained conserved throughout evolution. A search for homology (21) between this variable sequence and proteins in the National Biomedical Research Foundation library did not reveal any significant relationships with other known proteins.

Although sequence data are not available from the same carboxy-terminal portion of the human erythroid α-subunit, Speicher and Marchesi (30, 31) did note variability in repeat 10 and predicted that through gene duplication the carboxy-terminal domain might also vary. The carboxy-terminal domain of the human erythroid β-subunit, which contains phosphorylation sites, was also noted to vary from the regular internal repeat structure (30, 31). Thus, departure from the normal repetitive structural motif at terminal regions may be necessary for specialized functions, which tend to localize at terminal domains in most spectrin chains (16).

The chromosomal location of the nonerythroid α-spectrin sequences was determined by Southern blot analysis of DNA from 20 human × Chinese hamster somatic hybrid cell lines (9–12) with RBF2 as a hybridization probe (Fig. 4A). Three restriction fragments, 14, 5.6, and 2.1 kb in size, were detected in *Bgl*II digests of human DNA (Fig. 4A, lane 1). Chinese hamster DNA (lane 2) yielded fragments of 7, 4.5, 1.8, 1.4, and 0.9 kb. Only the two larger human fragments were scored in the hybrids. Both were present in all seven hybrids which had retained an intact copy of human chromosome 9. All other human chromosomes were ruled out by three or more discordant hybrids. In addition, two hybrids contained chromosome 9 with partially deleted short arms (missing region 9pter-p21). Both were still positive for the human nonerythroid α-spectrin sequences (Fig. 4A, lane 12). Reprobing the filter in Fig. 4 with the human nonerythroid α-spectrin cDNA NBF1 confirmed these results. These data indicate that the human nonerythroid α-spectrin locus (which is designated SPTAN1) lies within the region 9p21-qter.

Independent assignment was obtained by in situ hybridization (15) of clone RBF2 to human metaphase chromosomes. Of 127 silver grains scored over chromosomes in 50 cells, 25 (20%) were at chromosome 9, bands q33-q34 (Fig. 4B). No other significant peaks of hybridization were observed. These in situ hybridization results are consistent with the presence of a single gene for nonerythroid α-spectrin and refine its localization to the distal bands of the long arm of chromosome 9. At least seven other genes have been mapped to this region, including those for the ABO blood group (8) and the human homolog of the v-*abl* oncogene (17). Human erythrocyte α-spectrin had been previously assigned to a locus on chromosome 1, bands q22 to q25 (18). We have not detected any significant cross-hybridization with this site on chromosome 1 by our in situ and Southern blotting studies.

Evidence supporting the notion that erythroid and nonerythroid α-chains have diverged recently from a common gene, as suggested in early reports demonstrating immunological cross-reactivity (14, 16, 27), has been provided by detailed comparisons of the deduced nonerythroid peptide sequence with all available erythroid α-chain sequences (5, 30, 31). Thus, for example, erythroid α-spectrin repeat 19 (30, 31)

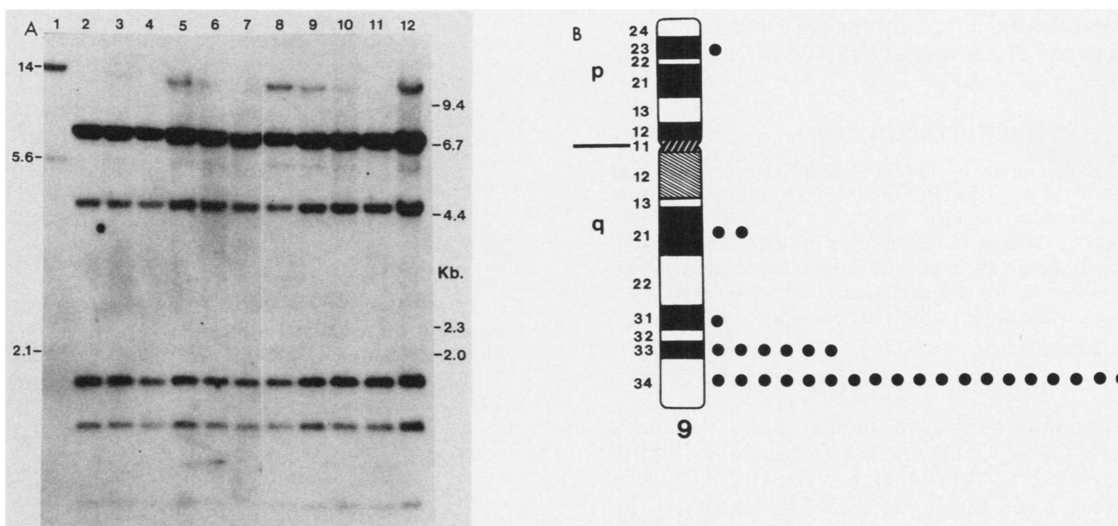

FIG. 4. Chromosomal mapping of the human nonerythroid α-spectrin gene. (A) Hybridization of ^{32}P-labeled (7) rat brain α-spectrin cDNA (RBF2) with *Bgl*II-digested DNA (10 μg per lane) from human × Chinese hamster hybrid cells and controls. Lanes: 1, human lymphoblastoid cells; 2, Chinese hamster DNA; 3, 5, 6, 8, 9, and 10, hybrid cell lines containing human chromosome 9; 4, 7, and 11, hybrid lines lacking human chromosome 9; 12, hybrid with deleted chromosome 9; only 9p21-qter is present. (B) Silver grain distribution along chromosome 9 (total from 50 cells) after in situ hybridization with clone RBF2 labeled by nick translation with [^{3}H]dCTP, [^{3}H]dTTP, and [^{3}H]dATP.

showed closest homology (51%) with repeat 19 of the nonerythroid α-chain. Likewise, repeats 16 and 17 of nonerythroid α-spectrin showed comparable homology with the analogous repeats deduced from the mouse and human erythroid cDNAs (5) aligned in Fig. 1. All other spectrin repeats showed significantly lower homology (<35%) with other nonanalogous α-fodrin repeats. Furthermore, interspecies homologies between erythroid and nonerythroid α-spectrins were consistent with their phylogenetic distances. Thus, on the basis of the comparison with erythroid α-spectrin sequences, which show a consistent pattern of homology with analogous repeats, the nonerythroid α-spectrin subunit is expected to contain 21 repeating units, with a variable sequence at the carboxy-terminal domain.

These findings document an extraordinary conservation among nonerythroid α-spectrins. In contrast, erythroid α-spectrin has diverged rapidly in mammals. These trends suggest interesting differences in functional roles for these proteins in erythroid and nonerythroid tissues. Although the nonerythroid α-spectrins interact with β-chains that vary in both size and function, the high degree of conservation in α-chains is indicative of strong structural and functional constraints common to all nonerythroid spectrins. These constraints have been drastically altered by the recent specialization of mammalian erythrocytes. These cells differ from other vertebrate erythrocytes in that they lack nuclei, as well as a number of other cytoskeletal components including microtubules and intermediate filaments. Thus, unlike avian erythrocytes, which express the nonerythroid α-subunit (27), mammalian erythrocytes, in the absence of other cytoskeletal structures, probably exert new adaptive pressures on α-spectrin structure and function. Human erythrocyte spectrin appears to be functionally quite distinct from the nonerythroid spectrins characterized thus far. Its divergent functional properties include the ability to self-associate into complex oligomers (16, 25), weaker subunit-subunit interactions (23), and loss of a calcium-dependent calmodulin-binding site on the α-subunit (29). These findings suggest that a rapid functional divergence among membrane skeletal proteins has occurred in mammalian erythrocytes in response to changing demands related to membrane mechanical strength and deformability. In this regard, it is interesting that mammalian erythrocytes vary considerably in size and shape.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants GM 21714 to V.T.M., GM 26105 to U.F., and AM 28076 to E.J.B. from the National Institutes of Health.

We are grateful to William C. Horne and Bernard G. Forget for reading this paper, David W. Speicher for peptide sequence data, and Lucy A. Godley, Jay W. Schneider, and Robert W. Mercer for technical assistance and advice provided throughout this work.

ADDENDUM IN PROOF

Since submission of this paper, another group, utilizing a fibroblast-derived α-fodrin cDNA, has assigned the α-fodrin gene to chromosome 1 (A. P. McMahon, D. H. Giebelhaus, J. E. Champion, J. A. Bailes, S. Lacy, B. Carritt, S. K. Henchman, and R. T. Moon, Differentiation 34:68–78, 1987). Sequence from the 5′ end of clone NBF1 (this report) aligns with 100% homology with the fibroblast cDNA (repeat 10 of clone 3). Probes from both NBF1 and the fibroblast clone 2.7a recognize the same restriction fragment length polymorphism. However, hybridization of probe 2.7a to 11 rodent × human hybrid DNAs digested with *Bgl*II revealed concordancy of human specific fragments with chromosome 9. A site of hybridization on chromosome 1 was excluded by four discordant hybrids.

LITERATURE CITED

1. **Barton, D. E., T. L. Yang-Feng, and U. Francke.** 1986. The human tyrosine aminotransferase gene mapped to the long arm of chromosome 16 (region 16q22 → q24) by somatic cell hybrid analysis and in situ hybridization. Hum. Genet. **72**:221–224.
2. **Bennett, V.** 1985. The membrane skeleton of human erythrocytes and its implications for more complex cells. Annu. Rev. Biochem. **54**:273–304.
3. **Bennett, V., J. Davis, and W. E. Fowler.** 1982. Brain spectrin, a membrane-associated protein related in structure and function to erythrocyte spectrin. Nature (London) **299**:126–131.
4. **Birkenmeier, C. S., D. M. Bodine, E. A. Repasky, D. M. Helfman, S. H. Hughes, and J. E. Barker.** 1985. Remarkable homology among the internal repeats of erythroid and nonerythroid spectrin. Proc. Natl. Acad. Sci. USA **82**:5671–5675.
5. **Curtis, P. J., A. Palumbo, J. Ming, P. Fraser, L. Cioe, P. Meo, S. Shane, and G. Rovera.** 1985. Sequence comparison of human and murine erythrocyte α-spectrin cDNA. Gene **36**:357–362.
6. **Dale, R. M. K., B. A. McClure, and J. P. Houchins.** 1985. A rapid single-stranded cloning strategy for producing a sequential series of overlapping clones for use in DNA sequencing: application to sequencing the corn mitochondrial 18S DNA. Plasmid **13**:31–40.
7. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
8. **Ferguson-Smith, M. A., D. A. Aitken, C. Turleau, and J. de Grouchy.** 1976. Localization of the human ABO: Np-1: AK-1 linkage group by regional assignment of AK-1 to 9q34. Hum. Genet. **34**:35–43.
9. **Francke, U.** 1984. Random X inactivation resulting in mosaic nullisomy of region Xp21.1 → p21.3 associated with heterozygosity for ornithine transcarbamylase deficiency and for chronic granulomatous disease. Cytogenet. Cell Genet. **38**:298–307.
10. **Francke, U., N. Busby, D. Shaw, S. Hansen, and M. G. Brown.** 1976. Intrachromosamal gene mapping in man: assignment of nucleoside phosphorylase to region 14cen → 14q21 by interspecific hybridization of cells with t[x;14] [p22;q21] translocation. Somatic Cell Genet. **2**:27–40.
11. **Francke, U., and B. Francke.** 1981. Requirement of the human chromosome 11 long arm for replication of herpes simplex virus type 1 in nonpermissive Chinese hamster × human diploid fibroblast hybrids. Somatic Cell Genet. **7**:171–191.
12. **Francke, U., and M. Pellegrino.** 1977. Assignment of the major histocompatibility complex to a region of the short arm of human chromosome 6. Proc. Natl. Acad. Sci. USA **74**:1147–1151, 5776.
13. **Glenney, J. R., Jr., P. Glenney, M. Osborne, and K. Weber.** 1982. An F-actin and calmodulin binding protein from isolated intestinal brush borders has a morphology related to spectrin. Cell **28**:843–854.
14. **Glenney, J. R., Jr., P. Glenney, and K. Weber.** 1982. Erythroid spectrin, brain fodrin, and intestinal brush border proteins (TW-260/240) are related molecules containing a common calmodulin binding subunit bound to a variant cell type-specific subunit. Proc. Natl. Acad. Sci. USA **79**:4002–4005.
15. **Harper, M. E., and G. F. Saunders.** 1981. Localization of single copy DNA sequences on G-banded human chromosomes by in situ hybridization. Chromosoma **83**:431–439.
16. **Harris, A. S., L. A. D. Green, K. J. Ainger, and J. S. Morrow.** 1985. Mechanism of cytoskeletal regulation (I): functional differences correlate with antigenic dissimilarity in human brain and erythrocyte spectrin. Biochim. Biophys. Acta **830**:147–158.
17. **Heisterkamp, N., J. Groffen, J. R. Stephenson, N. K. Spurr, P. N. Goodfellow, E. Solomon, B. Carritt, and W. F. Bodmer.** 1982. Chromosome localization of human cellular homologues

of two viral oncogenes. Nature (London) **299**:747–749.

18. **Huebner, K., A. P. Palumbo, M. Isobe, C. A. Kozak, S. Monaco, G. Rovera, C. M. Croce, and P. J. Curtis.** 1986. The β-spectrin gene is on chromosome 1 in mouse and man. Proc. Natl. Acad. Sci. USA **82**:3790–3793.
19. **Kohl, N. E., C. E. Gee, and F. W. Alt.** 1984. Activated expression of the N-*myc* gene in human neuroblastoma and related tumors. Science **226**:1335–1337.
20. **Levine, J., and M. Willard.** 1983. Redistribution of fodrin (a component of the cortical cytoplasm) accompanying capping of cell surface molecules. Proc. Natl. Acad. Sci. USA **80**:191–195.
21. **Lipman, D. J., and W. R. Pearson.** 1985. Rapid and sensitive protein similarity searches. Science **227**:1435–1441.
22. **Maizel, J. V., and R. P. Lenk.** 1981. Enhanced graphic matrix analysis of nucleic acid and protein sequences. Proc. Natl. Acad. Sci. USA **78**:7665–7669.
23. **Marchesi, V. T.** 1985. Stabilizing infrastructure of cell membranes. Annu. Rev. Cell Biol. **1**:531–561.
24. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–78.
25. **Morrow, J. S., and V. T. Marchesi.** 1981. Self-assembly of spectrin oligomers in vitro: a basis for a dynamic cytoskeleton. J. Cell Biol. **88**:463–468.
26. **Nelson, W. J., C. A. L. S. Calaco, and E. Lazarides.** 1983. Involvement of spectrin in cell surface receptor capping in lymphocytes. Proc. Natl. Acad. Sci. USA **80**:1626–1630.
27. **Repasky, E. A., B. L. Granger, and E. Lazarides.** 1982. Widespread occurrence of avian spectrin in nonerythroid cells. Cell **29**:821–833.
28. **Repasky, E. A., D. E. Symer, and R. B. Bankert.** 1984. Spectrin immunofluorescence distinguishes a population of naturally capped lymphocytes in situ. J. Cell Biol. **99**:350–355.
29. **Sears, D. E., V. T. Marchesi, and J. S. Morrow.** 1986. A calmodulin and β-subunit binding domain in human erythrocyte spectrin. Biochim. Biophys. Acta **870**:432–442.
30. **Speicher, D. W.** 1986. The present status of erythrocyte spectrin structure: the 106-residue repetitive structure is a basic feature of an entire class of proteins. J. Cell. Biochem. **30**:245–258.
31. **Speicher, D. W., and V. T. Marchesi.** 1984. Erythrocyte spectrin is comprised of many homologous triple helical segments. Nature (London) **311**:177–180.
32. **Wasernius, V.M., M. Saraste, J. Knowles, I. Virtanen, and V. P. Lehto.** 1985. Sequencing of the chicken nonerythroid spectrin cDNA reveals an internal repetitive structure homologous to the human erythrocyte spectrin. EMBO J. **4**:1425–1430.
33. **Young, R. A., and R. W. Davis.** 1983. Efficient isolation of genes by using antibody probes. Proc. Natl. Acad. Sci. USA **80**:1194–1198.
34. **Young, R. A., and R. W. Davis.** 1983. Yeast RNA polymease II genes: isolation with antibody probes. Science **222**:778–782.